Short communication

Differential actions and excitotoxicity of glutamate agonists on motoneurons in adult mouse cervical spinal cord slices

N. Hori\textsuperscript{a}, Y. Tan\textsuperscript{a}, M. King\textsuperscript{a}, N.L. Strominger\textsuperscript{a,b}, D.O. Carpenter\textsuperscript{a,*}

\textsuperscript{a}Department of Environmental Health, School of Public Health, University at Albany, 1 University Place, Rm. B242, Rensselaer, NY 12144-3456, USA

\textsuperscript{b}Albany Medical College, Albany, NY 12208, USA

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Abstract

Electrical activity was recorded from motoneurons in adult mouse cervical spinal cord (C4–8) slices. Motoneurons showed almost no response to ionophoretic application of N-methyl-D-aspartic acid (NMDA) in both control and Mg\textsuperscript{2+}-free media, but very sensitive to kainate (KA) and amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA). Bath perfusion of KA, AMPA or glucose/O\textsubscript{2} free Krebs–Ringer solution, but not bath perfusion of NMDA, caused membrane depolarization within 3 min and beading of the dendrite trees after more than 10 min perfusion. Our results indicate that adult motoneurons have few or no NMDA receptors.

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Until recently it has not been possible to produce an in vitro spinal cord slice preparation from an adult mammal that contains viable motoneurons. Although spinal motoneurons have been studied in dissociated cultures [16] and slice preparations from neonatal animals [1,3,12], only recently has any laboratory succeeded in maintaining viable motoneurons in a spinal cord slice from adult animals. Recently, Carlin et al. [5] has reported characteristics of ionic currents in functionally mature motoneurons in a slice, and we [9] have published methods for rat spinal cord slices from mature animals in which motoneurons are viable and apparently healthy.

In the rodent neonatal spinal cord motoneurons exhibit receptors for both NMDA and AMPA, and the properties of NMDA receptors are not unusual [1,4]. However, NMDA receptors gradually disappear postnatally. Binding studies have shown that while there is a high density of NMDA receptors during the first week of life, these are lost by the second or third postnatal week [14]. Since excitatory amino acid receptors play a critical role in neuronal cell death in motoneuronal diseases such as amyotrophic lateral sclerosis (ALS) and spinal cord injury, we have extended our previous observations on adult rat spinal cord to use of mouse spinal cord in order to exploit mouse models of neurologic disease.

Transverse cervical (C4–8) spinal cord slices were made from 40 male ICR mice weighing around 35 g (2–3 months of age) using methods similar to those previously reported for the rat [9]. Animals were deeply anesthetized with pentobarbitol (60 mg/kg) and perfused transcardially with 20 ml of cold (\textdegree{C}) oxygenated modified Krebs–Ringer solution (212.5 mM sucrose, 3.5 mM KCl, 2.4 mM CaCl\textsubscript{2}, 1.3 mM MgSO\textsubscript{4}, 26 mM NaHCO\textsubscript{3}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4} and 10 mM glucose) [2]. A laminectomy was performed to expose the cervical spinal cord. After exposure of the brainstem and midcollicular transection, a ligature was placed around the spinal cord near the medulla and it was slowly elevated with a manipulator while the dorsal and ventral roots were cut with a pair of ophthalmic micro-scissors under a Zeiss operating micro-
scope, with care being taken to avoid stretching of the roots. The spinal cord was removed from the medulla to T2. Throughout the dissection, the spinal cord was continuously bathed with oxygenated, cold modified Krebs–Ringer solution. A single block of tissue was sandwiched between two chilled agar blocks on the stage of a vibratome. Up to 12 transverse slices (450 μm) were cut from each preparation. Slices were incubated for a period of about 1 h in the oxygenated modified Krebs–Ringer solution at 34 °C and then transferred to normal Krebs–Ringer solution (125 mM NaCl, 3.5 mM KCl, 2.4 mM CaCl₂, 1.3 mM MgSO₄, 26 mM NaHCO₃, 1.2 mM KH₂PO₄ and 10 mM glucose) and incubated for at least an additional 30 min. Recordings were performed in a submerged slice recording chamber perfused with normal Krebs–Ringer solution circulating at about 3 ml/min at 34 °C [9].

The recording, stimulating and ionophoretic electrodes were placed as shown diagrammatically in Fig. 1A. Concentric tungsten stimulating electrodes with an exposed
tip diameter of 25 μm and length of 300 μm (Yni-ku-Medical, Japan) were positioned just within the spinal cord near the sites of emergence of the ventral and dorsal roots and in the dorsal funiculus at the site of the pyramidal tract for antidromic and orthodromic activation of the motoneurons, respectively. Intracellular current–clamp recordings were made with glass micropipettes filled with 3 M K-acetate (Fig. 1A). For morphological analysis, Lucifer Yellow CH (Sigma, 10% in distilled water) was injected into a cell through a microelectrode by application of 2 nA negative current pulses of 250 ms duration at 2 Hz for 1.5–2 min before application of amino acid agonists or transient ischemia. In some experiments, responses of motoneurons to ionophoretic application of excitatory amino acid agonists (AMPA, NMDA and KA at 10 mM in 0.15 M NaCl, pH 7.5), applied through a multi-barrel micropipette, were recorded.

Slices with apparently healthy motoneurons could be maintained for more than 12 h in vitro, and intracellular recordings could often be maintained for up to 3 h. The critical factors in obtaining viable motoneurons were: (1) minimizing mechanical tension during removal of the spinal cord, (2) slicing the cord while sandwiched between two chilled agar blocks, (3) using the sodium-free, modified Krebs–Ringer solution throughout the whole process of dissecting and cutting the cord, and (4) preincubating slices for 1 h in the modified Krebs–Ringer solution before transfer to a sodium-containing solution, as originally described by Aghajanian and Rasmussen [2].

Lucifer Yellow injection revealed normal spinal motoneuron morphology characterized by dendrites ramifying from the ventral horn into ipsilateral and sometimes also the contralateral dorsal horn. The axon is readily identifiable by its swollen cut end and projection into the region of the ventral root (arrow in Fig. 1B). Upon intracellular recording, the motoneurons were silent but could be identified by an antidromic response evoked by stimulation at the exit of the ventral root (VR, S2), an EPSP evoked by stimulation of the dorsal funiculus (DF, S1) at the site of the descending pyramidal tract and by stimulation of the dorsal horn (DH, S3) to activate muscle afferents. Spontaneous EPSPs and IPSPs also were present. The current–voltage (I–V) relationship, determined by passing current through the recording electrode through a bridge circuit, was approximately linear within ±15 mV from resting potential. Membrane potential was 65.8±4.4 mV and membrane resistance was 24.0±2.7 MΩ, respectively (n=10) (Fig. 1F).

Both AMPA and KA, but not NMDA, caused pronounced depolarization and firing when applied ionophoretically (Fig. 2A and B) or by superfusion (Fig. 2C). Perfusion with Mg²⁺-free Krebs–Ringer did not alter the lack of a significant NMDA response (Fig. 2B). The depolarization caused by bath application of AMPA (10⁻⁵ M, n=5) and KA (10⁻⁷M, n=4) became irreversible after about 7 min exposure. Bath application of NMDA (10⁻⁵M) either had no effect on membrane potential (n=4 of 6), or after 5 min caused a small hyperpolarization (n=2 of 6) (Fig. 2C) (presumably secondary to NMDA activation of inhibitory interneurons). Superfusion with glucose/O₂-free Krebs–Ringer, like AMPA and KA, caused depolarization, rapid firing and subsequent spike inactivation that became irreversible after several minutes. Concurrently, antidromically elicited action potentials decremented and disappeared (Fig. 2B).

To evaluate effects on cellular morphology, motoneurons were filled with Lucifer Yellow prior to exposure to glutamate agonists or ischemic conditions. Conditions that caused irreversible depolarization also resulted in prominent changes in structure. Exposure to AMPA, KA or simulated ischemia resulted in dendritic blebbing (n=more than 30), while NMDA had no effect (Fig. 3). Dendritic blebbing has been shown to be a sign of
Fig. 3. Morphology of motoneurons injected with Lucifer Yellow before perfusion with NMDA (10⁻³ M, A), AMPA (10⁻³ M, B), KA (10⁻³ M, C) for 7 min and glucose/O₂ free Krebs–Ringer solution (D) as an ischemia model. The dendritic changes should be compared to the control motoneuron shown in Fig. 1B. The morphologic changes are typical of results from at least eight neurons studied in each exposure condition.
injury preceding cell death caused by both ischemia [10] and excitotoxicity [19,22]. A variety of factors are known to contribute to ischemic and excitotoxic cell death, including disruption of ionic concentration gradients, loss of membrane transport systems, accumulation in intracellular calcium and mitochondrial dysfunction [6,13,20,23,24].

As in adult rat spinal motoneurons [9], we find an almost complete absence of NMDA receptors on motoneurons in the adult mouse. This is consistent with the reported loss of NMDA receptors in the spinal cord postnatally [12] and contrasts with the depolarizing actions of NMDA on neonatal spinal motoneurons [1,21], where NMDA has been reported to induce rhythmic activity [18]. These results stand in contrast to the important roles of NMDA receptors higher in the neuroaxis, where NMDA receptors play a central role in both ischemic [10,11,15] and excitotoxic [25] cell death, and have been implicated in the etiology of several human diseases [17]. There is evidence for the presence of NMDA receptors on hypoglossal motoneurons in the adult rat [7], which raises the possibility that cranial and spinal motoneurons express a different profile of excitatory amino acid receptors. It is also interesting that NMDA receptors do exist on adult frog motoneurons [8].

It appears that the mechanisms of neuronal cell death by injury and in disease may differ between the brain and spinal cord. There is considerable potential value of the adult mouse spinal cord slice for study of some of the transgenic and knockout mice that are available models of human motoneuronal diseases. However, our results suggest that NMDA receptors will not play the important role in etiology of these diseases that might be expected from results obtained from more cephalic levels of the nervous system.

References


